OPTIMIZATION AND SCALE-UP OF CELL CULTURE AND PURIFICATION PROCESSES FOR PRODUCTION OF AN ADENOVIRUS-VECTORED TUBERCULOSIS VACCINE CANDIDATE

Chun Fang Shen, National Research Council of Canada
chunfang.shen@cnrc-nrc.gc.ca
Danielle Jacob, National Research Council of Canada
Tao Zhu, Tianjin CanSino Biotechnology Inc., China
Alice Bernier, National Research Council of Canada
Zhongqi Shao, Tianjin CanSino Biotechnology Inc., China
Xuefeng Yu, Tianjin CanSino Biotechnology Inc., China
Mehul Patel, National Research Council of Canada
Stephane Lanthier, National Research Council of Canada
Sven Ansorge, National Research Council of Canada
Amine Kamen, National Research Council of Canada & McGill University

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Tuberculosis (TB) is the second leading cause of death by infectious disease worldwide. The only available TB vaccine is the Bacille Calmette-Guerin (BCG). However, parenterally administered *Mycobacterium bovis* BCG vaccine confers only limited immune protection from pulmonary tuberculosis in humans. There is a need for developing effective boosting vaccination strategies. AdAg85A, an adenoviral vector expressing the mycobacterial protein Ag85A, is a new tuberculosis vaccine candidate, and has shown promising results in pre-clinical studies and phase I trial. This adenovirus vectored vaccine is produced using HEK 293 cell culture.

Here we report on the optimization of cell culture conditions, scale-up of production and purification of the AdAg85A at different scales. Four commercial serum-free media were evaluated under various conditions for supporting the growth of HEK293 cell and production of AdAg85A. A culturing strategy was employed to take advantages of two culture media with respective strengths in supporting the cell growth and virus production, which enabled to maintain virus productivity at higher cell densities and resulted in more than two folds of increases in culture titer. The production of AdAg85A was successfully scaled up and validated at 60L bioreactor under the optimal conditions.

The AdAg85A generated from the 3L and 60L bioreactor runs was purified through several purification steps. More than 98% of total cellular proteins was removed, over 60% of viral particles was recovered after the purification process, and purity of AdAg85A was similar to that of the ATCC VR-1516 Ad5 standard. Vaccination of mice with the purified AdAg85A demonstrated a very good level of Ag85A-specific antibody responses. The optimized production and purification conditions were transferred to a GMP facility for manufacturing of AdAg85A for generation of clinical grade material to support clinical trials.